A STUDY OF WOOD PRESERVATIVE LEACHATES FROM DOCKS IN AN ESTUARINE ENVIRONMENT

by

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ABSTRACT

In this study, we examined the concentrations and biological effects of certain metals and organic compounds that are typically found in wood preservatives used to protect dock pilings, bulkheads, and other wooden structures from decay. Our study focused on leachates from private docks in South Carolina macrotidal creek systems. Copper, chromium, arsenic, and polynuclear aromatic hydrocarbons (PAHs) were measured in composite samples of surficial sediments and naturally occurring oyster populations (*Crassostrea virginica*) from creeks with high densities of docks, and from nearby reference creeks with no docks. Sediments from all but one site had metal and total PAH concentrations which were below levels reported to cause biological effects. Solid-phase Microtox[7] bioassays using whole sediments and rotifer bioassays using sediment pore water showed no significant differences in acute toxicity between creeks with and without docks. Oysters growing directly on dock pilings had significantly higher concentrations of copper than oysters growing at least 10 m away; however, there was no significant difference in the physiological condition of these oysters. Four-day field bioassays measuring percent survival of mummichogs (*Fundulus heteroclitus*), mud snails (*Ilyanassa obsoleta*), juvenile red drum (*Sciaenops ocellatus*), and juvenile white shrimp (*Penaeus setiferus*) showed no significant differences between sites near to and distant from newly constructed docks. Hatchery-reared oysters showed no significant differences between dock and reference sites in percent survival, growth, or bioaccumulation of metals after six weeks of exposure. Our results suggest that, in macrotidal estuarine environments, wood preservative leachates from dock pilings have no acutely toxic effects on four common estuarine species, nor do they affect the survival or growth of juvenile oysters over a six-week time period. In some cases, metal leachates may accumulate in sediments and oysters immediately adjacent to pilings, but do not appear to become concentrated in sediments or oysters elsewhere in the same creeks.

INTRODUCTION

The proliferation of residential docks in coastal South Carolina has spawned a growing debate over their cumulative environmental impact. Until recently, relatively little information on the effects of docks was available to guide the decisions of governmental agencies charged with permitting or commenting on their construction. In the past few years, however, evidence has suggested that the wood preservative most commonly used in dock pilings and bulkheads (chromated copper arsenate or CCA) can be toxic to several estuarine organisms in controlled laboratory experiments (Weis and Weis, 1992b; Weis *et al.*, 1991; Weis *et al.* 1992). The relevance of these findings has been disputed, however, in a critique prepared on behalf of the American Wood Preservers Institute (Breteler, 1992). Criticism of the Weis's research focused primarily on the high wood surface to water volume ratio, and the intermittently static conditions under which their experiments were conducted. Breteler (1992) claimed that the Weis's experiments exaggerated the magnitude and duration of exposure to leachates experienced by organisms in natural estuarine environments. In support of Breteler's argument, Baldwin et al. (1994) presented evidence in the annual proceedings of the American Wood Preservers' Association that the percent survival of amphipods (*Ampelisca abdita*) was actually higher in sediments mixed with leachates from CCA-treated piles than it was in sediments mixed with leachates from untreated wood. The authors maintained that their test protocol more closely approximated conditions in the natural environment than that of the Weis's studies, and concluded that leaching of metals from properly fixed CCA-treated wood does not occur at concentrations which would adversely affect the survival of this species.

Subsequent to their laboratory investigations, however, Weis et al.(1993a) conducted field studies which demonstrated that oysters growing on treated wood in a bulkheaded canal had higher metal concentrations in their soft tissue and a greater prevalence of a histopathological disorder than oysters collected from nearby rocks. The authors also reported that concentrations of metals, particularly copper, were elevated in fine-grained sediments adjacent to CCA-treated piles and bulkheads (Weis et al., 1993c), as well as in other organisms living on or near these structures (Weis and Weis, 1992b; 1993; Weis et al., 1993b). In field experiments, Weis and Weis (1992a; 1994) found that the biomass and diversity of sessile epifaunal communities were significantly lower on treated wood panels than on untreated wood panels; although, this effect was shown to

dissipate over time and become negligible after three months of exposure.

The study described herein was intended to complement the earlier investigations of Weis et al., in an effort to determine whether wood preservative leachates from residential docks are acutely toxic to representative estuarine species in natural tidal creek systems (rather than in the laboratory or in heavily bulkheaded canals). Our primary objective was to evaluate the environmental effects of CCA leachates; however, we also examined concentrations of polynuclear aromatic hydrocarbons (PAHs) in sediments and oysters near to and distant from docks. These organic compounds are major components of creosote, a wood preservative which was formerly used more frequently than CCA to treat dock pilings. Creosote contaminated sediments have been shown to have both lethal and sublethal effects on several species of estuarine fish and invertebrates (Chmura and Ross, 1978; Roberts *et al*., 1989; Rostad and Pereira, 1987; Sami et al., 1992; Swartz *et al*., 1989; Tagatz *et al*., 1983; Vogelbein *et al*., 1990). The toxicity of this compound has been traced to several of its component PAHs, many of which have been shown to have carcinogenic, teratinogenic or mutagenic effects (Long and Morgan, 1990). PAHs are also major components of other fossil fuel products associated with boating activity, such as motor oil, gasoline, and engine exhaust.

Our study was conducted in two phases. Specific objectives of each phase were as follows:

Phase I:

1) compare concentrations of copper (Cu), chromium (Cr), arsenic (As), and PAHs in sediments and oysters from creeks with and without high densities of docks;

2) compare the physiological condition and shell thickness of oysters from creeks with and without docks;

Phase II:

1) compare the survival, growth, and bioaccumulation of metals (Cu, Cr, and As) and PAHs in laboratory-reared oysters placed near to and distant from newly constructed docks for a period of six weeks;

2) compare the survival of several species of estuarine fishes and invertebrates placed near to and distant from newly constructed docks for a period of four days.

METHODS

Phase I:

Study Sites: Ten tidal creeks with high densities of docks and ten nearby reference creeks with no docks (Fig. 1) were selected for study. These creeks were located throughout the meso- and polyhaline reaches of the Charleston Harbor Estuary and nearby drainage systems. Creeks with high dock densities included two branches of Hobcaw Creek, Boone Hall Creek, Molasses Creek, Orangegrove Creek, two branches of James Island Creek, one branch of Parrot Creek, Oak Island Creek, and Horseshoe Creek. Dock densities in these creeks ranged from approximately 9-18 docks per kilometer of shoreline, as measured from the mouth of each creek (or creek branch) to its upper navigable limit at high tide. Reference creeks, many of which are unnamed, were selected on the basis of their similarity in geographic location, depth, width, and salinity regime to creeks with high dock densities.

Field Sampling and Sample Preparation: Field sampling for Phase I was conducted in late February and early March of 1994, prior to the annual spawning of oysters. Field surveys indicate that this is the period during which uptake and accumulation of PAHs and other lipophilic compounds reaches a maximum in oysters, coinciding with the seasonal storage of glycogen and lipids in preparation for spawning (Marcus and Stokes, 1985). Late fall through early spring is also the period during which oysters are harvested for human consumption, and thus can pose a health threat if contaminated by pollutants.

In each of the ten creeks with docks, we collected one 0.5-l surficial sediment sample (to a depth of approximately 8 cm) from the lower intertidal zone immediately adjacent to each of five randomly selected docks. Samples from all five dock sites were then combined to form a single composite sediment sample for each creek. A second composite sediment sample was collected in a similar manner from five lower intertidal sites in the same creek, but at least 10 m from any dock. A third composite sediment sample was collected from approximately the same tidal elevation in a nearby reference creek. This sampling procedure resulted in our collecting a total of 30 composite sediment samples, each of which was homogenized in the field and subdivided for chemical, grain size, and toxicity analyses. Sediments were collected with solvent-rinsed stainless steel spatulas, transferred to pre-rinsed glass jars with teflon lids, and placed on ice. In the laboratory, sediments were stored under refrigeration at 4°C until chemical analyses were performed. All chemical analyses were completed within 30 days of collection.

In each of the creeks with high dock densities, one composite oyster sample (consisting of about 50 oysters) was collected directly from pilings of the same five docks where sediments had been sampled. A second composite oyster sample was collected from one or more natural intertidal beds located in the same creek, but at least 10 m from any dock. A third composite oyster sample was collected from five intertidal sites of similar elevation in the corresponding reference creek. This protocol resulted in the collection of 30 composite oyster samples, each of which was then subdivided for chemical and condition index analyses. In the laboratory, the external shell surface of each oyster was scrubbed and rinsed. Twenty oysters of harvestable size (>7.3 cm shell ht) were selected from each composite sample and refrigerated at 4°C until condition index analyses were performed (generally, within 48 hrs of collection). The remaining 30 oysters were placed in filtered seawater overnight to enable them to purge any sediments from their shell cavity. The oysters were then shucked and frozen until they were analyzed for metals and PAHs. All extractions for PAH analyses were completed within 30 days of collection.

Sediment Composition: Percentages of sand, silt, and clay were determined for each composite sediment sample using pipette analysis following procedures described by Plumb (1981). Approximately 20 g of sediment were homogenized, weighed, and wet-seived with distilled water through a 63-F seive. Material remaining on the seive was dryed at 90°C for approximately 8 hrs and weighed as the sand fraction. Dispersant (sodium hexametaphosphate) and distilled water were added to the filtrate in a graduated cylinder, which was then stoppered and inverted several times to suspend the sediment particles. Total silt and clay fractions were determined by extracting 20-ml aliquots at the appropriate depths and time intervals, and drying the extracts overnight at 90°C. Percentages of sand, silt, and clay were calculated as proportions of the total dry weight of the sample.

Contaminants Analyses: For each sediment and oyster tissue sample, approximately 5 g (wet wt) was acid digested using concentrated HNO_3, concentrated HCL, and 30% hydrogen peroxide following a modification of EPA Method 200.3 (USEPA, 1991). Samples were then analyzed for copper, chromium, and aluminum using Inductively Coupled Plasma Spectrometry (ICP) following EPA method 200.7 (USEPA, 1991) with a Varian Liberty 200 ICP system. Samples were analyzed for arsenic using graphite furnace atomic absorption spectrophotometry following EPA method 200.9 (USEPA, 1991) with a Perkin-Elmer spectrometer (Model Z-5100 or ZL-4100). Quality control procedures included the analysis of duplicate, blank, and matrix spiked

samples (one per ten samples), as well as NIST Standard Reference Materials (SRM 1646 Estuarine Sediment and SRM 1566a Oyster Tissue).

Sediments and oysters were also analyzed for 12 PAHs, using a modification of EPA method 525.1. Samples were extracted with a mixture of methanol, isopropanol, and acetone. A 15% solution of the filtered extract was then passed through a 500-mg C18 extraction column that had been cleaned with successive rinses of methylene chloride, hexane, ethyl ether, methanol, and 15% extraction solvent. After drying, the C18 column was eluted with methylene chloride to a volume of 1.0 ml. The sample was then analyzed on a Tracor model 540 gas chromatograph with a nonpolar (J&W DB-5) capillary column and a flame ionization detector. Quality control procedures included the analysis of duplicate, blank, and spiked samples.

Concentrations of copper, chromium, and arsenic in sediments were normalized with respect to aluminum levels, in order to correct for differences among sites related to sediment type and natural watershed differences. This analytical method, described by Windom *et al.*(1989), is based on the fact that concentrations of trace metals in sediments covary with concentrations of natural aluminosilicate minerals (i.e., clays) which, in turn, are associated with fine-grain sediments. After $\log_{10}$-transforming all trace metal concentrations, a linear regression of Cu, Cr, or As versus Al was performed for the ten reference site samples. After calculating the 95% prediction interval for the sample population, metal concentrations for all of the other sites were plotted as a function of aluminum. Those values falling outside the 95% prediction interval were considered to be elevated with respect to the reference sites.

Concentrations of copper, chromium, and arsenic in sediments were also compared among site groups using analysis of covariance (ANCOVA), in which aluminum was the covariate. Concentrations of Cu, Cr, and As in oyster tissue were compared among site groups using one-way analysis of variance (ANOVA) after $\log_{10}$-transforming the data. Concentrations of total PAHs in both sediments and oysters were compared among site groups using one-way ANOVA applied to $\log_{10}$-transformed data.

Microtox Bioassays: Solid-phase tests were conducted according to standardized protocols with the Microtox Model 500 system (Bulich, 1979; Ross et al., 1991; Microbics Corp., 1992a). The Microtox bioassay has been found to be a sensitive indicator of acute toxicity for a variety of chemical compounds (Bulich, 1979; Bulich and Isenberg, 1981; De Zwart and Sloof, 1983; Ross and Henebry, 1989; Schiewe et al., 1985). For each of the 30 sampling sites, one 0.3-g

sediment sample was used to make a series of 12 dilutions ranging from 0.01% to 10% sediment, which were then incubated with cultures of the bioluminescent bacterium *Photobacterium phosphoreum* for 20 minutes. A column filter was then used to separate the liquid phase containing the bacteria from the sediment, and the post-exposure light output of the bacteria was measured. After correcting for natural light decay in control cultures, the decrease in light production of exposed cultures was plotted as a dose-response curve. A linear regression model was then used to calculate the EC_{50} value for each sample (i.e., the sediment concentration required to reduce luminescence by 50%). Differences in EC_{50} values among site groups were compared using one-way ANOVA, after $\log_{10}$-transforming the data. Dock densities (no. docks/km shoreline) and dry weight concentrations of sediment contaminants (Cu, Cr, As, and PAHs) were correlated with corrected EC_{50} values using the Spearman Rank Correlation Coefficient. Corrected EC_{50} values were calculated as described in the manufacturer's recommended protocol (Microbics Corp., 1992b), using the following formula:

$$EC_{50\ (corrected)} = EC_{50\ (initial)} - (EC_{50\ (initial)} \times \%\ water)$$

where, % water is expressed as a proportion of the total sediment sample weight.

Rotifer Bioassays: The acute toxicity of sediment pore water obtained from each of the composite sediment samples was evaluated using the rotifer *Brachionus plicatilis*. Approximately 30 ml of pore water was extracted from each sample using a syringe suction apparatus described by Winger and Lasier (1994). The pore water was then filtered through a 1ì glass filter to remove suspended particles and stored at 4°C. Immediately prior to testing, the pore water was brought to room temperature (25°C) and aerated for one minute. All rotifer bioassays were conducted within 30 days of sample collection and within 7 days of pore water extraction.

Replicate groups of 30 *B. plicatilis* were exposed to a 100% concentration of the pore water for 24 hrs using a modification of procedures described by Snell and Persoone (1989). Rotifer cysts, artificial seawater hatching medium, and multiwell test plates were obtained from Rototox-M**7** test kits. The rotifer cysts were hatched and grown for approximately 28 hrs in 2.5 ml of 20 ppt seawater under constant light. Thirty neonate rotifers were then transferred to 5 test wells (6 rotifers per well) containing 0.3 ml of the test medium after temporarily being placed in a rinsing trough containing 0.7 ml of the test medium. The rinsing trough minimized any dilution effects related to transfer of the growing medium along with the rotifers. The test plates were then covered and incubated in the dark for 24 hrs at 25°C. For each creek system, test plates included three

groups of test wells (5 wells per group) containing pore water from the three composite sediment samples (< 1 m from docks, > 10 m from docks, and reference site), and three groups of test wells containing 20 ppt seawater as a control. A reference toxicant test was conducted in conjunction with each test series using serial dilutions of the toxicant $K_2Cr_2O_7$. Percent survival was assessed in all treatment groups after 24 hrs, and results were compared among site groups using one-way ANOVA applied to arcsin-transformed data.

Oyster Condition Index and Shell Thickness Analyses: Twenty oysters (>7.3 cm shell ht) were selected from each composite sample for analysis of physiological condition. After all oysters were scrubbed clean of fouling organisms, shell height and wet weight were measured for each oyster. Shell height was measured from the tip of the umbo to the advancing edge of the ventral margin. The oysters were then shucked, and the soft tissue was dried at 80°C for approximately 48 hrs, or until a constant weight was obtained. Condition index (CI) was calculated for each oyster using the method of Lawrence and Scott (1982):

CI = (dry meat wt (g)/internal shell cavity capacity (g)) x 100

where, internal shell cavity capacity = total whole live weight (g) - dry shell weight (g).

This index has been used as an indicator of nutritive status and physiological stress by several other investigators, as well (Abbe and Sanders, 1988; Marcus et al., 1989; Wendt et al., 1990; Van Dolah et al., 1992). Condition index values were compared among site groups (near docks, away from docks, and reference sites) using the Kruskal-Wallis Test. Shell thickness was calculated using the method described by Frazier (1976), in which the weight of dry shell (mg) was divided by the shell surface area (SA), which was estimated by Galtsoff's (1964) formula: $SA = 2.5\ ht^{1.56}$, where ht is the shell height (cm). Shell thickness was compared among site groups using one-way ANOVA applied to $\log_{10}$-transformed data. Separate correlation analyses were performed for condition index and shell thickness versus tissue concentrations of Cu, Cr, and As using the Spearman Rank Correlation Coefficient.

Phase II:

Study Sites: In the second phase of our study, five newly constructed docks (4-12 mo old) and five nearby reference sites were selected for our 4-day *in situ* bioassays and our 6-week study of metal bioaccumulation and growth in oysters (Fig. 1). Among the five docks, three were located in New Cut Creek and two were located in Parrot Creek. Average salinities in both creeks

were in the polyhaline range. The corresponding reference sites were located within the same creeks, but at least 100 m from any dock. For this study phase, we purposely chose docks heads which were separated from upland areas by broad expanses of vegetated salt marsh in order to minimize any effects of nonpoint source pollution from residential runoff.

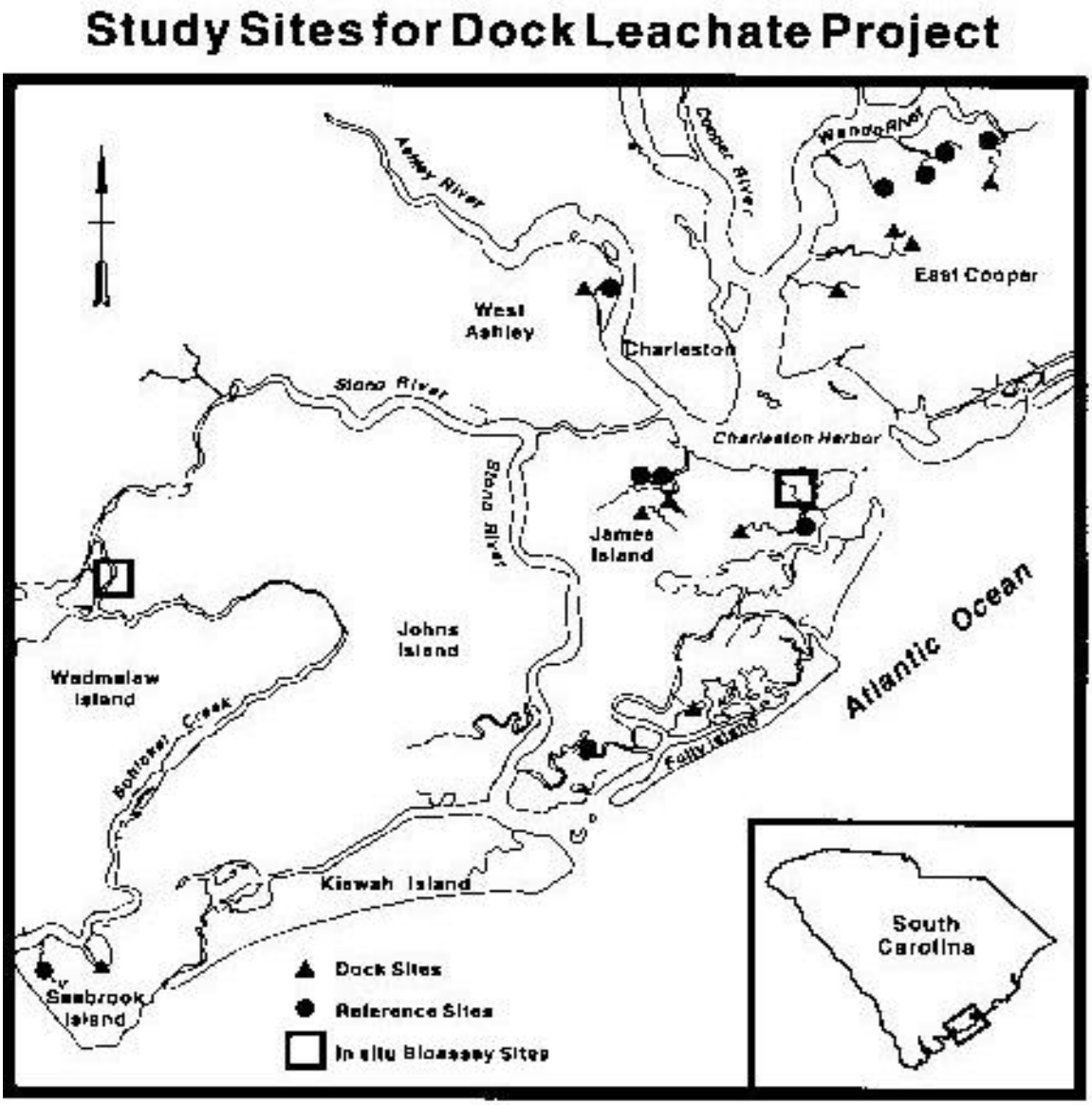

Figure 1. Study sites for wood preservative leachate project.

Four-Day *In Situ* Bioassays: Laboratory-reared stocks of juvenile white shrimp (*Penaeus setiferus*) and juvenile red drum (*Sciaenops ocellatus*), as well as field-collected mummichogs (*Fundulus heteroclitus*) and mud snails *(Ilyanassa obsoleta)* were used in our short-term field bioassays. At each dock site, 20 mummichogs (5-8 cm total length (TL)), 20 red drum (4-6 cm TL), and 20 white shrimp (6-8 cm TL) were placed separately in three modified plastic minnow traps (one per species), and suspended above the creek bottom from a dock piling. In addition, 50 mud snails (*Ilyanassa obsoleta*) were placed in one PVC core (7.5 cm i.d.), which was partially screened on all sides to permit the free flow of water and allow contact between the snails and the ambient sediments. At reference sites, traps and cores containing the same four species were tethered to stakes in the lower intertidal zone. After four days of exposure, the survivors in each container were counted and placed on ice for future contaminants analyses. During the course of the 4-day bioassays, temperature, salinity, pH, and dissolved oxygen were recorded every 30 min in each of the two creeks with a Hydrolab Datasonde-3 data logger.

Six-Week Oyster Growth and Bioaccumulation Studies: Hatchery-reared juvenile oysters (*Crassostrea virginica*) were individually tagged, weighed, and measured (shell height and width). Sixty oysters (2-3 cm shell ht) were placed in each of 10 plastic mesh cages, five of which were placed in the lower intertidal zone adjacent to the newly constructed docks, and five of which were placed at nearby reference sites located at least 100 m from any dock. Each cage was suspended horizontally, approximately 15-30 cm above the creek bottom to minimize the effects of siltation on growth and survival. Percent survival, growth, and bioaccumulation of metals were measured after six weeks.

RESULTS

Phase I:

Sediment Composition and Contaminants Analyses: Percentages of fine-grained sediments (silts and clays) ranged widely among our study sites, from a minimum of 37% in Hobcaw Creek to a maximum of 98% in Orangegrove Creek. The average silt/clay content of sediments was slightly higher for reference sites (Table 1); however, there was no consistent pattern in sediment composition with respect to dock proximity, and mean percentages of silts and clays did not differ significantly among the three site groups (<1 m from docks, >10 m from docks, and reference sites)(ANOVA, $p>0.05$).

Table 1. Mean percentages of sand, silt, clay, and silt+clay (±1 s.e.) in composite sediment samples from each of the three site groups.

Site Group	% Sand	% Silt	% Clay	% Silt/Clay
<1 m from Docks (n=10)	24.2 (±5.2)	19.1 (±1.7)	56.7 (±3.9)	75.8 (±5.2)
>10 m from Docks (n=10)	25.6 (±6.6)	19.7 (±2.4)	54.7 (±4.8)	74.4 (±6.6)
Reference (n=10)	19.1 (±6.8)	29.7 (±7.8)	51.2 (±7.2)	80.9 (±6.8)

n = number of replicate samples

Average copper concentrations in sediments ranged from approximately 19 to 58 ppm (dry wt) throughout the study area (Table 2). The highest concentration (401 ppm dry wt) occurred in one of two composite sediment samples taken from sites immediately adjacent to dock pilings in James Island Creek. This value exceeded Long and Morgan's (1990) ER-L ("Effects Range - Low") and ER-M ("Effects Range - Median") levels of 70 and 390 ppm (dry wt), respectively. With the exception of this one site, however, copper concentrations were less than 38 ppm, regardless of dock proximity. Furthermore, there were no statistically significant differences in mean copper concentrations among any of the three site groups, after adjusting for differences related to aluminum levels (ANCOVA, $p>0.05$). Only the James Island dock site had a copper concentration that exceeded the 95% prediction interval based on a regression of copper versus aluminum levels in the ten reference site sediment samples (Fig. 2).

Table 2. Mean concentrations of copper, chromium, arsenic, and total PAHs (±1s.e.) in composite sediment samples from each of the three site groups.

SEDIMENTS				
Site Group	**Copper (ug/g dry wt)**	**Chromium (ug/g dry wt)**	**Arsenic (ug/g dry wt)**	**Total PAHs[1] (ng/g dry wt)**
<1 m from Docks (n=10)	57.7 (±34.4)	41.1 (±3.2)	17.0 (±1.4)	978.3 (±346.4)
>10 m from Docks (n=10)	19.1 (±2.1)	32.4 (±3.4)	13.9 (±1.4)	690.0 (±294.3)
Reference (n=10)	19.8 (±2.7)	34.8 (±2.6)	16.5 (±2.4)	1183.8 (±324.7)

n = number of replicate samples

[1] Includes the following 12 PAHs: acenaphathalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, and benzo(a)pyrene.

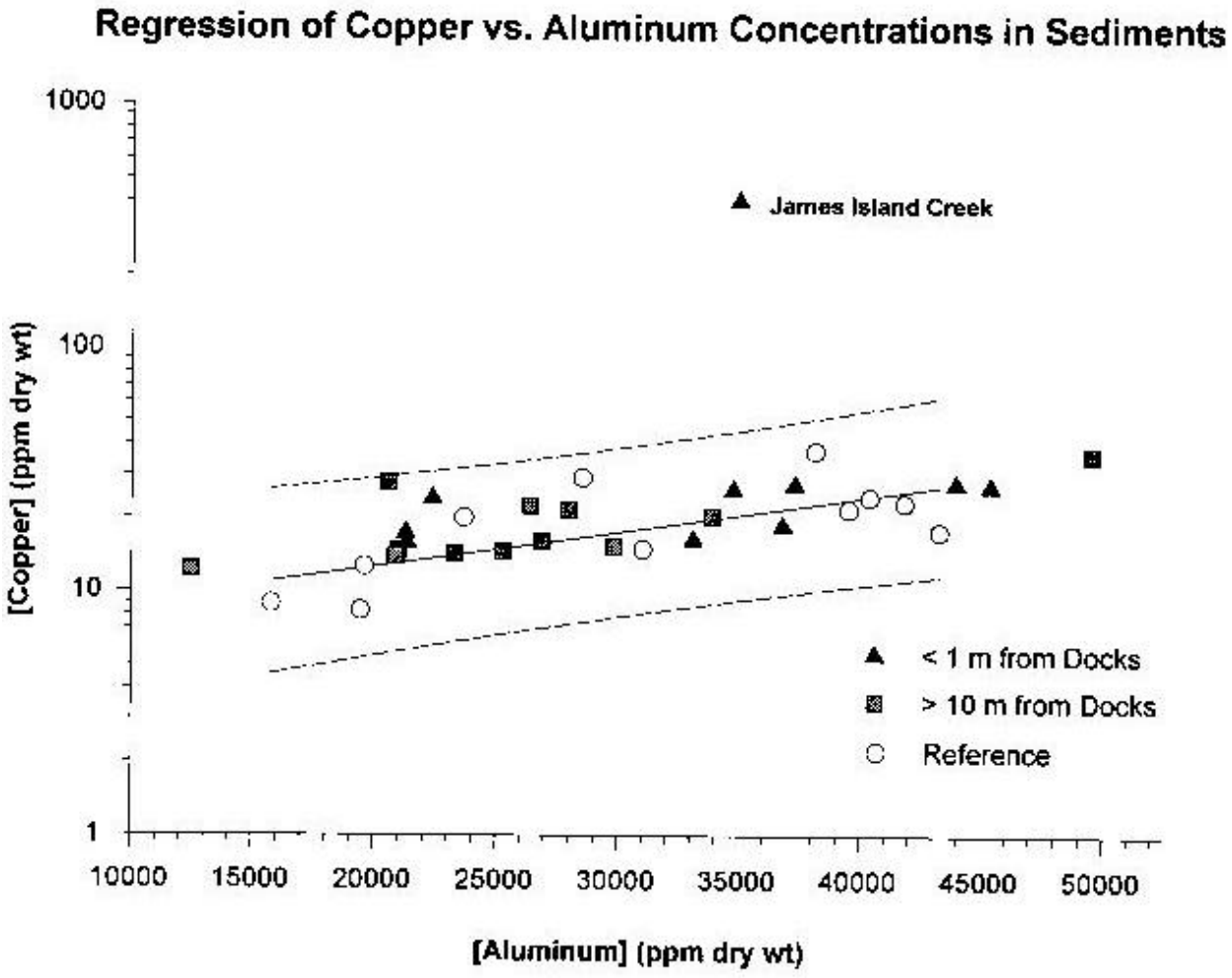

Figure 2. Copper versus aluminum concentrations (ppm dry wt) in sediments. Linear regression (solid line) and 95% prediction limits (dashed lines) based on Reference sediments, only).

Concentrations of chromium ranged from 21 to 64 ppm among all sites sampled, and were below the reported ER-L level of 80 ppm (Long and Morgan, 1990). Chromium concentrations were also lower than those reported for three National Status and Trends (NS&T) monitoring sites in Charleston Harbor (NOAA, 1991). After correcting for differences in aluminum levels, there were no significant differences in mean chromium concentrations among sites (ANCOVA, $p>0.05$;

Table 2). One composite sample taken near dock pilings in Molasses Creek had a normalized chromium concentration which slightly exceeded the 95% prediction interval for the regression of chromium versus aluminum (Fig. 3); however, this value was at the high end of the range measured, where the prediction interval is a less reliable indicator of natural variability in metal/aluminum ratios.

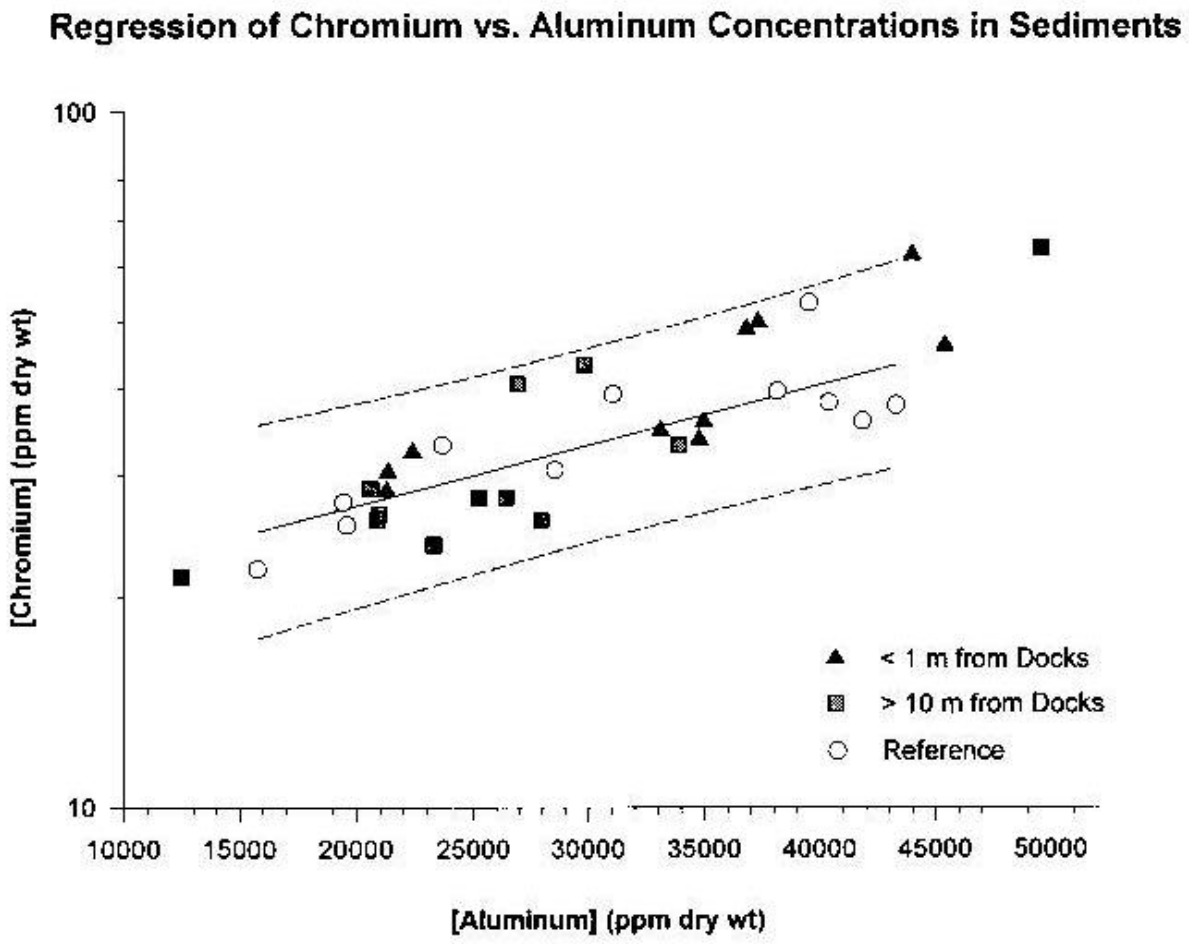

Figure 3. Chromium versus aluminum concentrations (ppm dry wt) in sediments. Linear regression (solid line) and 95% prediction limits (dashed lines) based on Reference sediments, only).

Arsenic concentrations in sediments ranged from 6 to 26 ppm throughout the study area. All concentratons were within the range reported for the three NS&T monitoring sites in Charleston Harbor (NOAA, 1991), and were below Long and Morgan's (1990) ER-L level of 33 ppm. After correcting for differences in aluminum levels, there were no statistically significant differences in mean arsenic concentrations among site groups (ANCOVA, $p>0.05$; Table 2). Four of the 20 samples from creeks with high dock densities had arsenic concentrations which slightly exceeded the 95% prediction interval for sediments having comparable aluminum levels (Fig. 4). Among these four samples, only one was collected immediately adjacent to dock pilings, however.

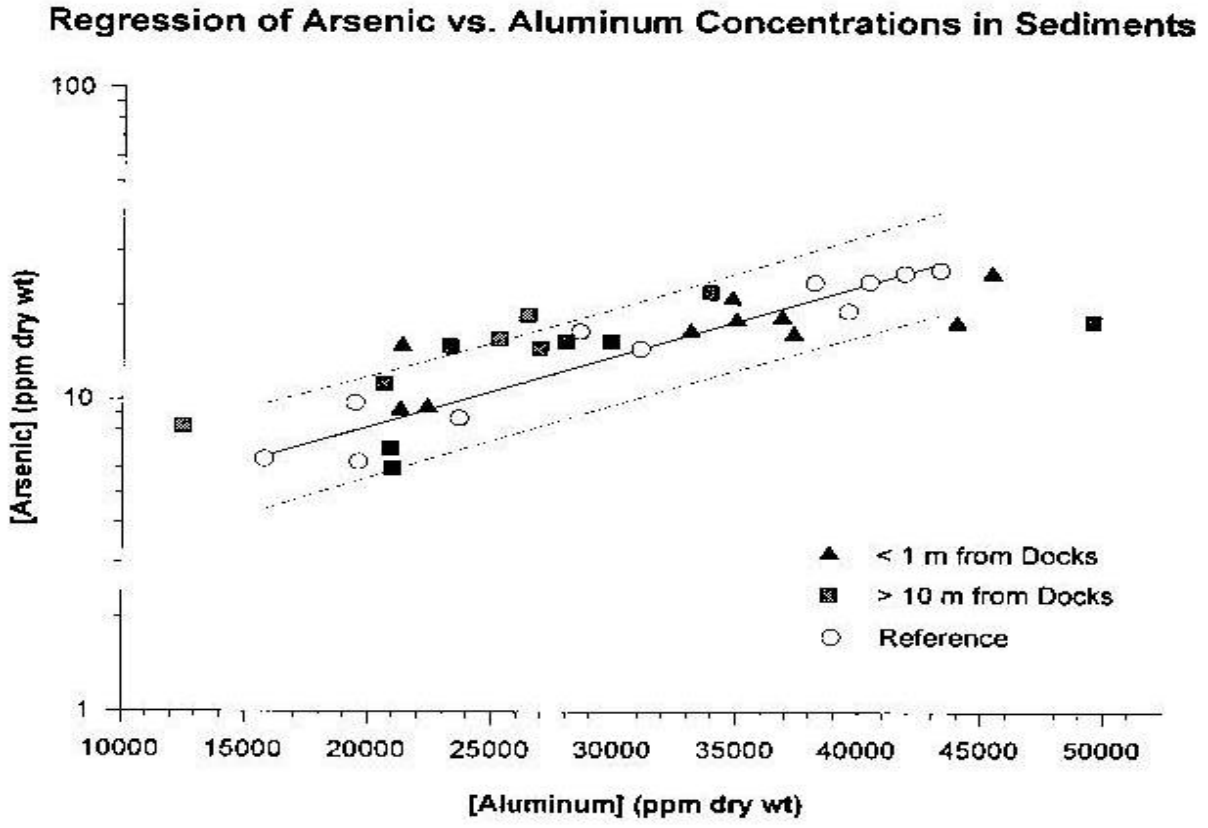

Figure 4. Arsenic versus aluminum concentrations (ppm dry wt) in sediments. (Linear regression (solid line) and 95% prediction limits (dashed lines) based on Reference sediments, only).

Concentrations of the 12 polynuclear aromatic hydrocarbons (PAHs) measured varied widely, both within and among site groups (Table 2). Several sites had concentrations of one or more PAHs which exceeded Long and Morgan's (1990) ER-L or ER-M levels; however, concentrations of total PAHs were below the ER-L level of 4000 ppb (dry wt) at all sites, regardless of dock proximity. There were no significant differences in total PAH concentrations among site groups (ANOVA, $p>0.05$). All three site groups had total PAH concentrations which exceeded those reported for two out of three NS&T sites in coastal South Carolina (NOAA, 1991), but were generally within the range of mean values reported for three nearby commercial marinas (Marcus and Stokes, 1985; Marcus et al., 1988).

Table 3. Mean concentrations of copper, chromium, arsenic, and total PAHs (±1s.e.) in composite oyster samples from each of the three site groups.

OYSTERS

Site Group	Copper (ug/g dry wt)	Chromium (ug/g dry wt)	Arsenic (ug/g dry wt)	Total PAHs[1] (ng/g dry wt)
<1 m from Docks (n=10)	**226.0* (±46.1)**	ND	8.3 (±1.1)	3547.3 (±1070.7)
>10 m from Docks (n=10)	134.4 (±38.3)	1.5 (±1.5)	7.6 (±0.9)	2057.6 (±1637.9)
Reference (n=10)	115.5 (±20.4)	ND	8.4 (±1.3)	2173.1 (±1960.5)

n = number of replicate samples

[1] includes the following 12 PAHs: acenaphathalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, and benzo(a)pyrene.

* indicates mean value is significantly different from other two means at $p<0.05$ (ANOVA, Duncan's Multiple Range Test).

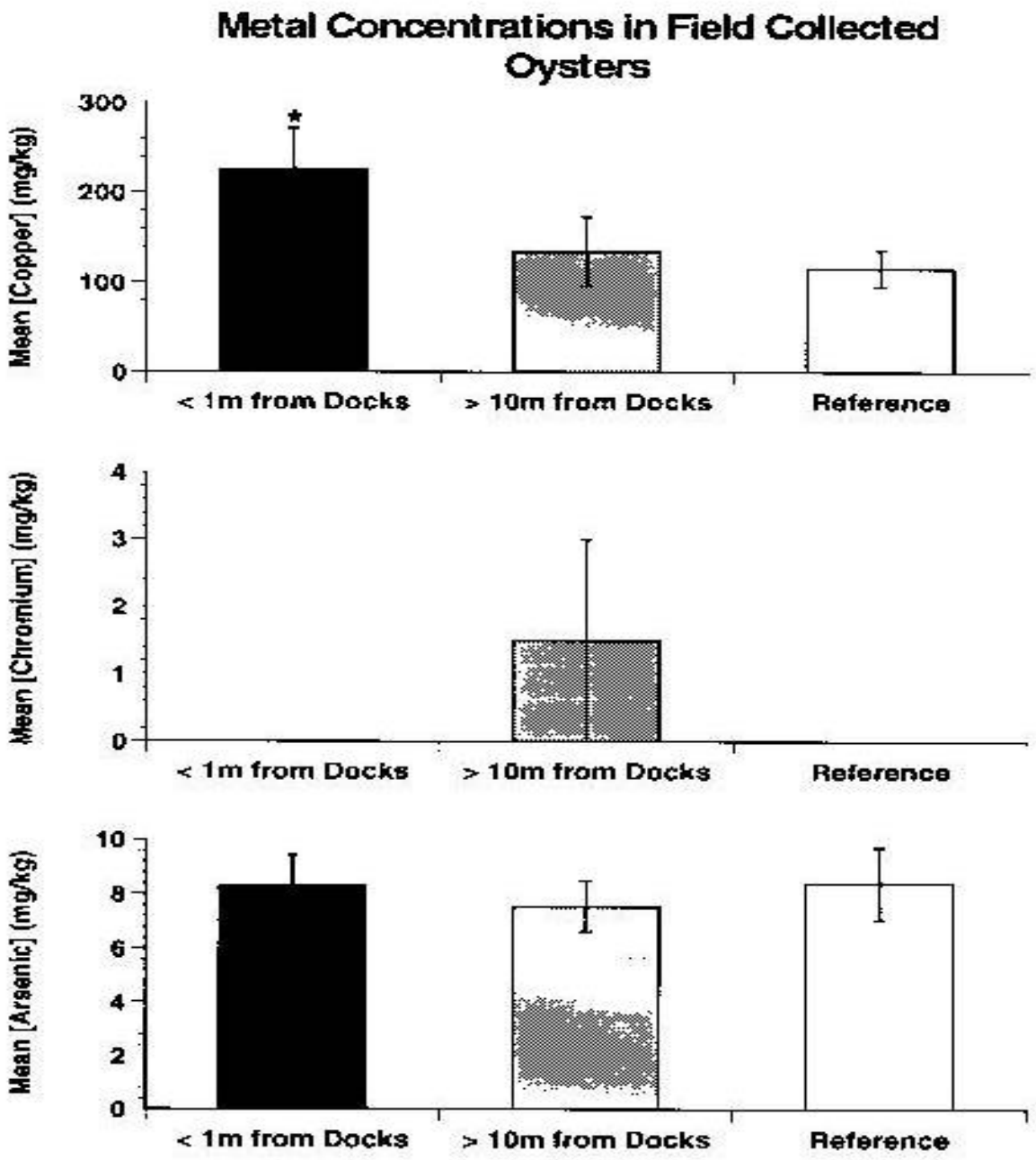

Figure 5. Mean (± 1 standard error) concentrations of copper, chromium, and arsenic (ppm dry wt) in composite oyster samples from each of the three site groups. (For each site group, n = 10 samples of 20 oysters each).

Oyster Contaminants Analyses: Copper concentrations in field-collected oysters ranged from 39 to 540 ppm dry wt (or approximately 8 to 108 ppm wet wt). Oysters collected from dock pilings had a significantly higher mean copper concentration (226 ppm dry wt; 45 ppm wet wt) than oysters collected from elsewhere in the same creeks, or oysters from reference creeks (ANOVA, $p<0.05$) (Table 3; Fig. 5). Copper concentrations in oysters from most sites were within the range of values reported for oysters from other sites throughout coastal South Carolina and Georgia (Mathews et al., 1979; Windom and Smith, 1972); however, copper levels in oysters from four sites approached or exceeded the maximum levels reported in these earlier studies (Fig. 6). Three of the four sites were immediately adjacent to dock pilings, while the fourth site was at least 10 m from any dock (Fig. 5).

There were no obvious patterns related to docks in mean tissue concentrations of chromium or arsenic (Table 3; Fig. 5). Chromium concentrations were below detection limits (<1 ppm) in all except one composite oyster sample, which was taken from natural intertidal beds located at least 10 m from any dock in Horseshoe Creek. Average arsenic concentrations were similar at all sites, ranging from 7.6 to 8.4 ppm (dry wt). These concentrations were well below average arsenic concentrations reported for oysters collected over a three-year period at two National Status and Trends (NS&T) monitoring sites in Charleston Harbor (NOAA, 1989).

Differences in total PAH concentrations in oysters from the three site groups were not statistically significant (ANOVA, $p>0.05$); however, the low power of the test (0.23 on a scale of 0 to 1) indicates that the likelihood of detecting a significant difference among site groups was small, given the large variance-to-mean ratio and the relatively small number of sites sampled. Mean concentrations of total PAHs ranged from 2058 ppb dry wt (412 ppb wet wt) in oysters collected from natural intertidal beds located at least 10 m from any dock, to 3547 ppb dry wt (709 ppb wet wt) in oysters collected directly from dock pilings (Table 3). Oysters from reference creeks had intermediate levels of these compounds (2173 ppb dry wt; 435 ppb wet wt). Total PAHs in oysters from dock pilings exceeded mean concentrations reported for oysters collected at two sites in Charleston Harbor (729 - 2670 ppb dry wt) during the first three years of the NS&T Program (NOAA, 1987); however, several of the component PAHs analyzed differed between the two studies, making meaningful comparisons of total PAHs difficult. Total PAHs in oysters from all three site groups also exceeded mean concentrations reported for oysters collected during the same season from three coastal marinas in South Carolina (<1-202 ppb wet wt) (Marcus and Stokes, 1985).

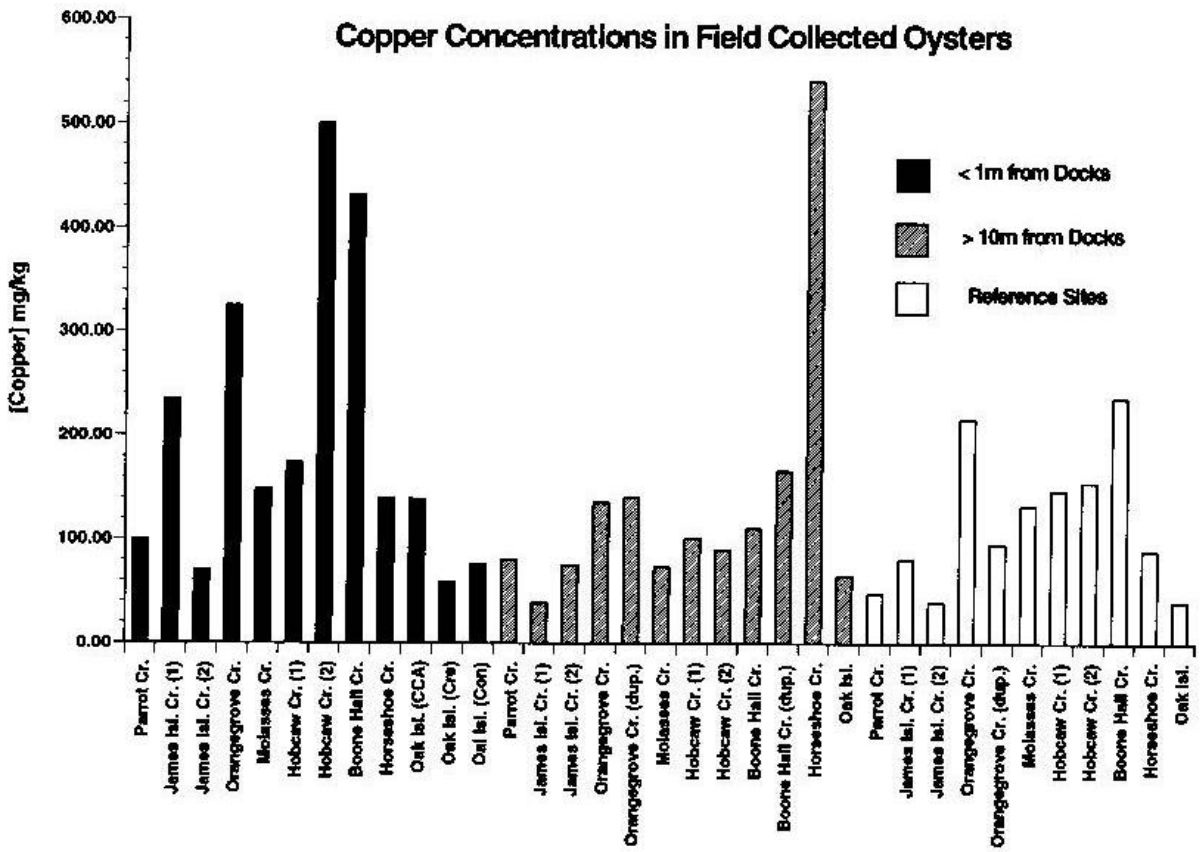

Figure 6. Copper concentrations (ppm dry wt) in composite oyster samples (including duplicates) from each of the 30 sampling sites. (For each site, n = 1 composite sample of 20 oysters; concentrations of copper in CCA-treated (CCA), creosoted (CRE), and concrete (CON) pilings from Oak Island Creek (OI01) reported separately for comparison.)

Oyster Condition Index and Shell Thickness Analyses: The physiological condition of field-collected oysters did not differ significantly among site groups (Kruskal-Wallis, $p>0.05$). Mean condition index (CI) values ranged from 7.88 for oysters from the ten reference sites to 8.01 for oysters from the ten dock sites (Fig. 7). These values were comparable to those reported by Van Dolah et al. (1992) for oysters collected from intertidal beds at marina and

reference sites in coastal South Carolina during February and March; however, they were lower than those reported for oysters collected during the same season from three other South Carolina marinas, where values ranged from 10.2 to 11.7 (Marcus et al., 1989). Mean shell thickness (dry shell weight/unit area) was slightly lower among oysters collected from dock pilings than it was from natural intertidal beds (Fig. 8); however, this difference was not statistically significant (ANOVA; $p>0.05$).

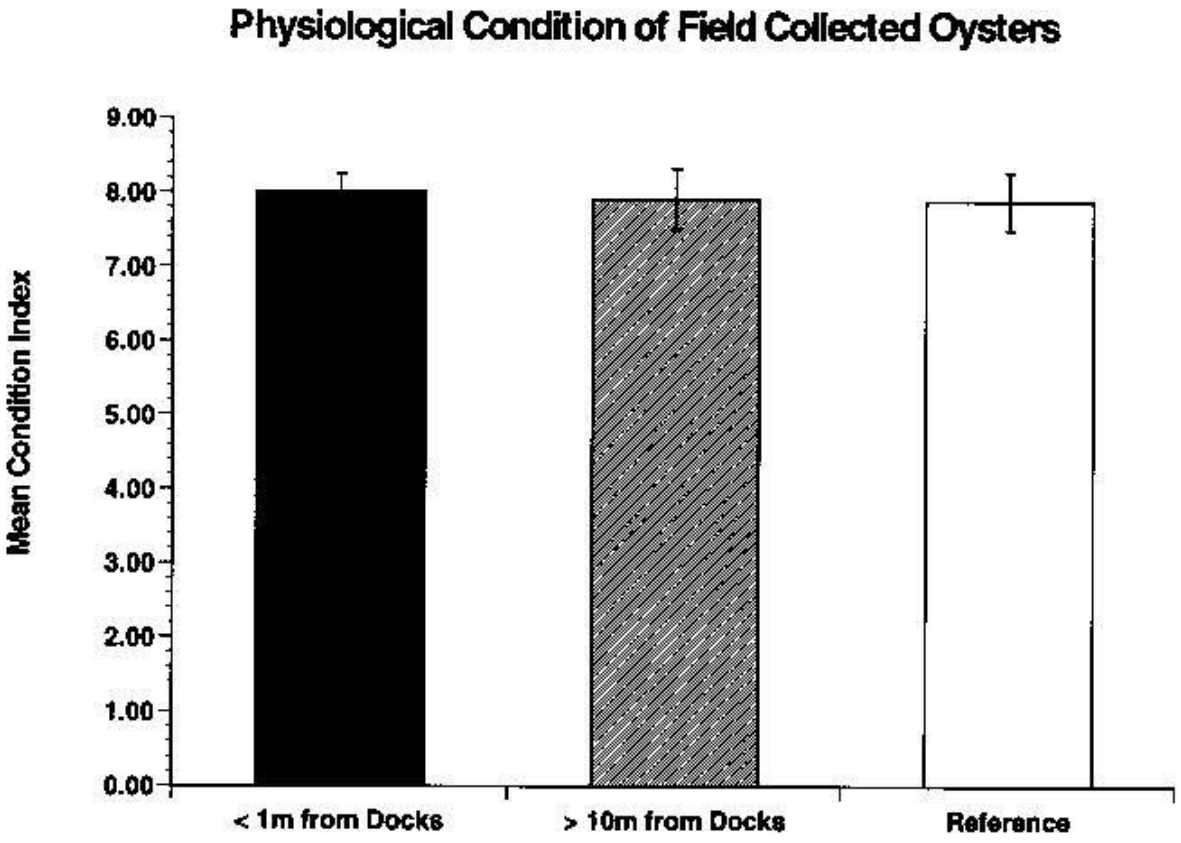

Figure 7. Mean (± 1 standard error) condition index values for oysters in composite samples from each of the three site groups. (For each site group, n = 10 samples of 20 oysters each).

There were no significant correlations between either condition index or shell thickness and copper concentrations in oyster tissue (Spearman Rank Correlation Coefficient; $p>0.05$); however both biological parameters were significantly correlated with arsenic levels (Table 4). Arsenic concentrations in oyster tissue were not correlated with dock density, however, suggesting that elevated levels of arsenic were unrelated to wood preservative leachates from docks. Previously published data indicate that oysters from several sites throughout the southeastern U.S., including two Charleston Harbor NS&T monitoring sites, are among those with the highest tissue concentrations of arsenic (NOAA, 1987). A similar enrichment of southeastern estuarine sediments by arsenic has been noted by Windom et al. (1989), who speculated that the prevalence of phosphate deposits throughout the region may account for the elevated arsenic levels.

Table 4. Matrix of Spearman Rank Correlation Coefficients relating dock density and oyster variables measured in study Phase I.

	OYSTERS					
	Copper	**Chromium**	**Arsenic**	**PAHs**	**Condition Index**	**Shell Thickness**
Chromium	0.30 (n=32)					
Arsenic	-0.26 (n=32)	0.24 (n=32)				
PAHs	0.25 (n=31)	0.04 (n=31)	-0.18 (n=31)			
Condition Index	0.07 (n=32)	0.28 (n=32)	**0.54* (n=32)**	0.07 (n=31)		
Shell Thickness	0.28 (n=32)	0.03 (n=32)	**-0.64* (n=32)**	0.30 (n=31)	-0.12 (n=32)	
Dock Density	0.13 (n=9)	ND	-0.24 (n=9)	-0.04 (n=9)	-0.51 (n=9)	-0.36 (n=9)

n = number of replicate samples

***** = significance at $p \leq 0.05$

ND = all concentrations below detection limits for 9 dock sites

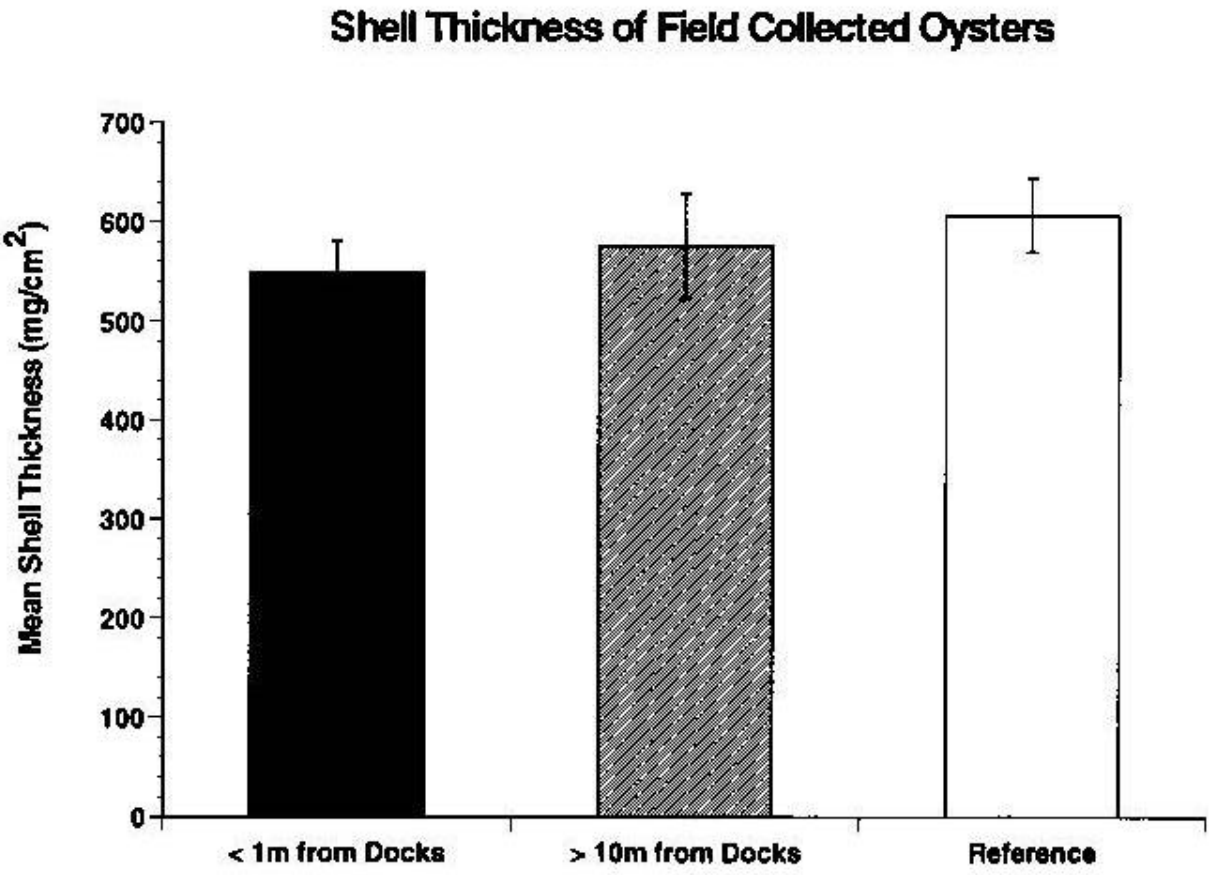

Figure 8. Mean (± 1 standard error) shell thickness of oysters in composite samples from each of the three site groups. (For each site group, n = 10 samples of 20 oysters each).

Microtox Bioassays: Solid-phase Microtox bioassays showed no significant difference in mean EC_{50} values among the three site groups (ANOVA, $p>0.05$) (Fig. 9). Results indicated that sediments were most toxic to *P. phosphoreum* in James Island Creek immediately adjacent to dock pilings (EC_{50}=0.08), and least toxic in Boone Hall Creek at sites located at least 10 m from any dock piling (EC_{50}=1.02). Significant negative correlations were found between corrected EC_{50} values (see methods) and dry weight concentrations of copper and arsenic (Spearman Rank Correlation Coefficient, $p<0.05$) (Table 5). This indicates that high concentrations of these two

metals are associated with high toxicity to *P. phosphoreum*. Corrected EC_{50} values for sites immediately adjacent to docks were negatively correlated with dock density (no. docks/km shoreline), indicating that the higher the dock density, the higher the sediment toxicity. It is important to note, however, that dock density was not significantly correlated with copper, chromium, or arsenic concentrations, suggesting that the relationship between sediment toxicity and dock density may be indicative of other anthropogenic stresses related to the density of adjacent residential development, rather than to the presence of docks themselves. Finally, we found a significant positive correlation between corrected EC_{50} values and total PAH concentrations in the sediments, suggesting that PAH levels were not acutely toxic to *P. phosphoreum*.

Table 5. Matrix of Spearman Rank Correlation Coefficients relating dock densities and sediment variables measured in study Phase I.

	SEDIMENTS				
	Copper	**Chromium**	**Arsenic**	**PAHs**	**$EC_{50(corr)}$**
Chromium	**0.60* (n=30)**				
Arsenic	**0.60* (n=30)**	**0.62* (n=30)**			
PAHs	-0.31 (n=29)	0.06 (n=29)	-0.05 (n=29)		
$EC_{50(corr)}$	**-0.41* (n=30)**	-0.14 (n=30)	**-0.42* (n=30)**	**0.38* (n=29)**	
Dock Density	0.56 (n=9)	-0.03 (n=9)	0.40 (n=9)	-0.53 (n=9)	**-0.74* (n=9)**

n = number of replicate samples

* = significance at $p \leq 0.05$

Rotifer Bioassays: The 24-hr rotifer bioassays showed no significant mortality for *Brachionus plicatilis* when the neonate rotifers were exposed to sediment pore water from any of our 30 sampling sites, regardless of dock proximity (Fig. 10). This finding is consistent with the

results of Microtox bioassays on bulk sediment samples (see above). Although metal concentrations in the pore water samples were not measured, our results suggest that copper levels were below the 24-hr LC_{50} value of 0.12 mg l^{-1} reported by Snell and Persoone (1989). Our results also suggest that pore water concentrations of chromium (VI), the most toxic form of the element, were below the 24-hr LC_{50} value of 115 mg l^{-1} reported for the batch of rotifer cysts provided with the Rototox test kits.

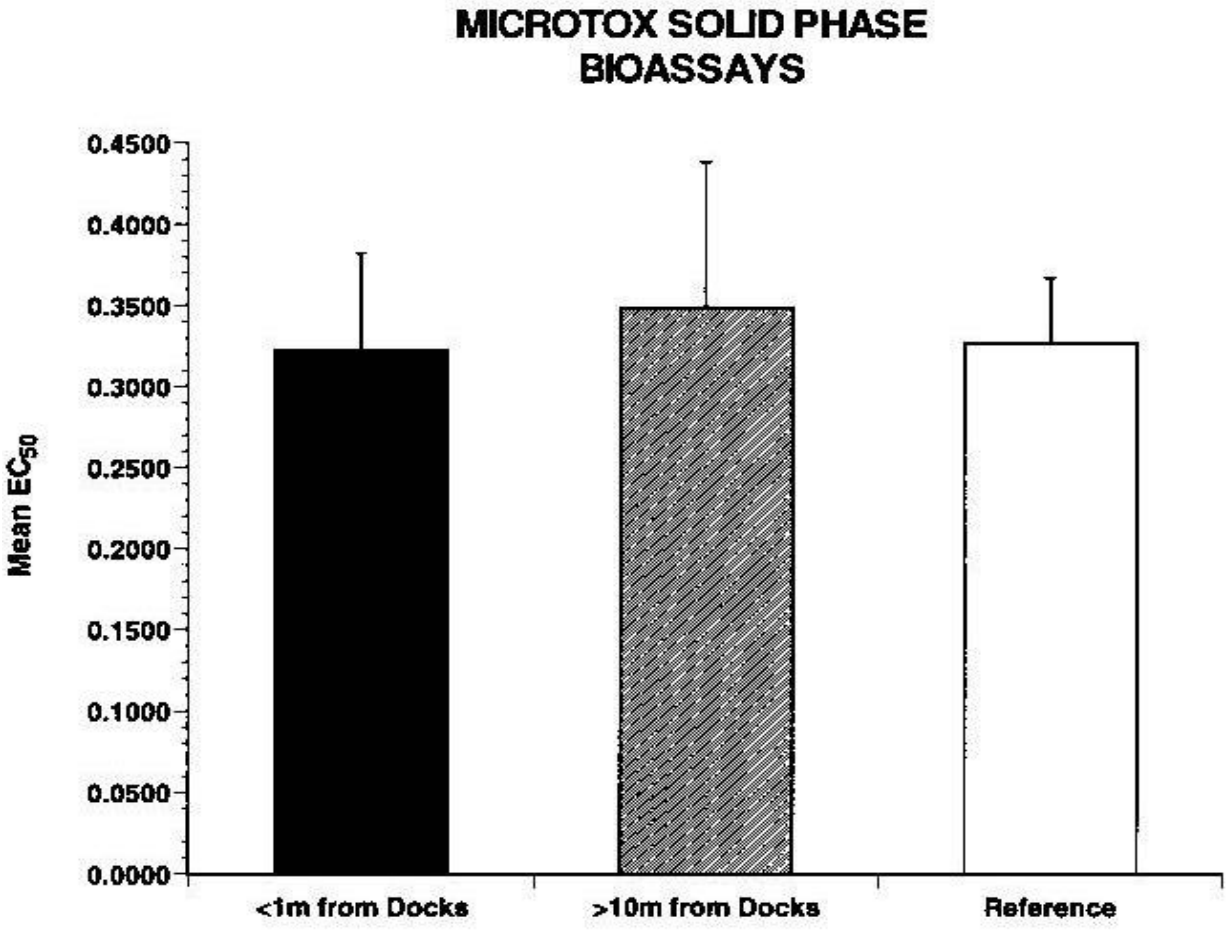

Figure 9. Mean (± 1 standard error) EC_{50} values for Microtox bioassays conducted on bulk sediment samples from each of the three site groups. (For each site group, n = 10 samples from 5 locations in each creek).

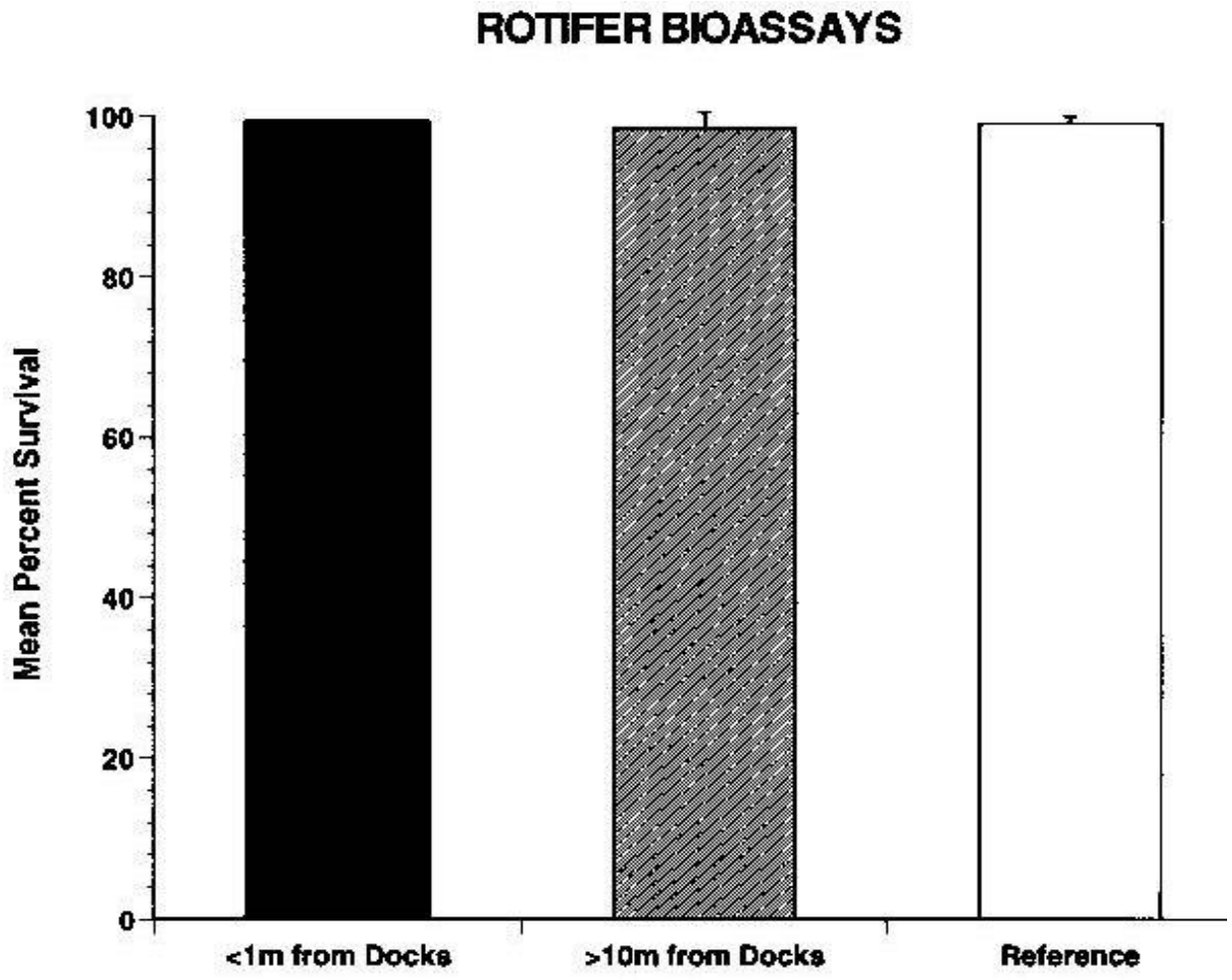

Figure 10. Mean (± 1 standard error) percent survival of rotifers after a 24-h exposure to pore water from composite sediment samples from each of the three site groups. (For each site group, n = 10 samples from 5 locations in each creek).

Phase II:

Four-Day *In-Situ* Bioassays: After four days of field exposure in Parrot and New Cut Creeks, there were no significant differences in mean percent survival of mud snails (*Ilyanassa obsoleta*), mud minnows (*Fundulus heteroclitus*), juvenile red drum (*Sciaenops ocellatus*), or

juvenile white shrimp (*Penaeus setiferus*) between sites near to and distant from newly constructed docks (Fig. 11). In addition, there were no significant differences between dock and reference sites with respect to mean concentrations of copper, chromium, or arsenic in composite sediment or tissue samples (ANOVA, $p>0.05$).

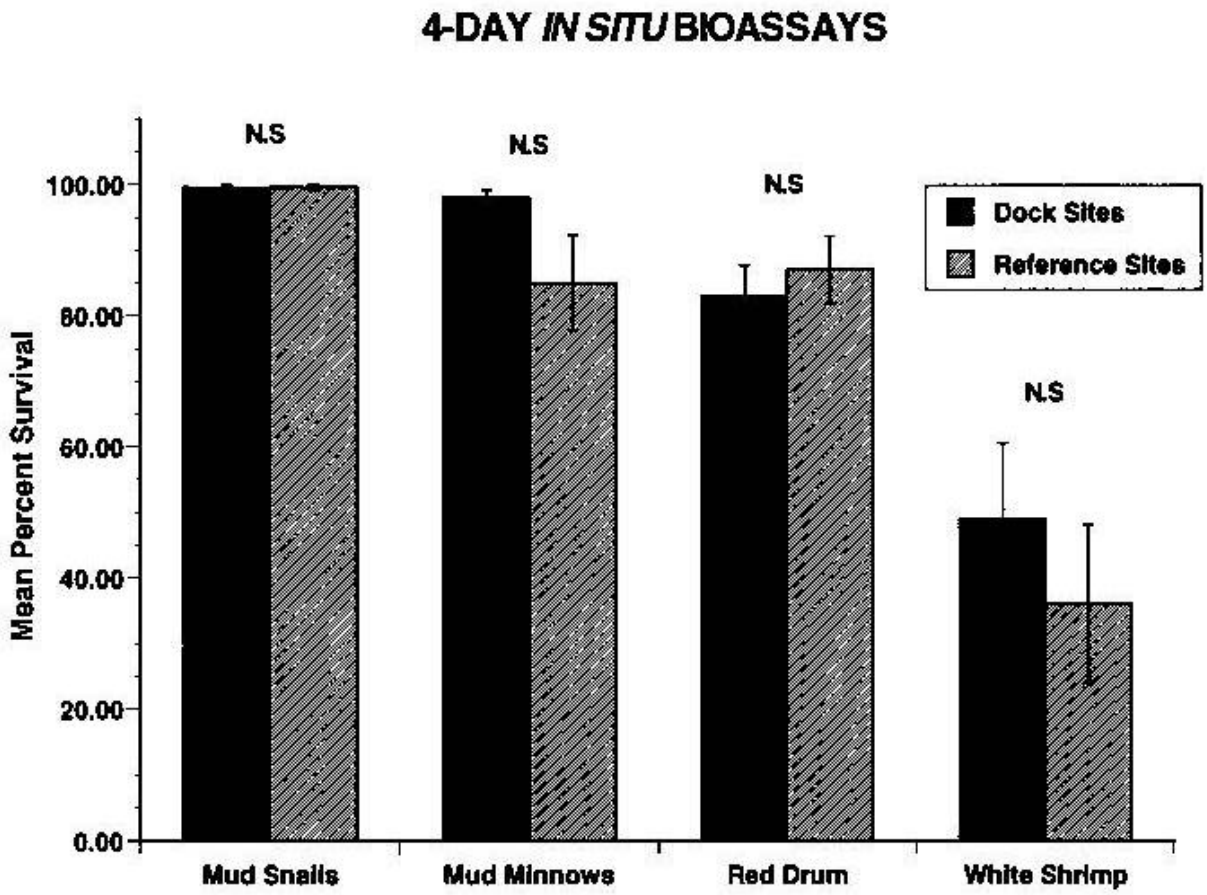

Figure 11. Mean (± 1 standard error) percent survival of mud snails, mud minnows, red drum, and white shrimp after 4-day *in situ* bioassays conducted near and away from exposure to pore water from composite sediment samples from each of the three site groups. (For each site group, n = 10 samples from 5 locations in each creek).

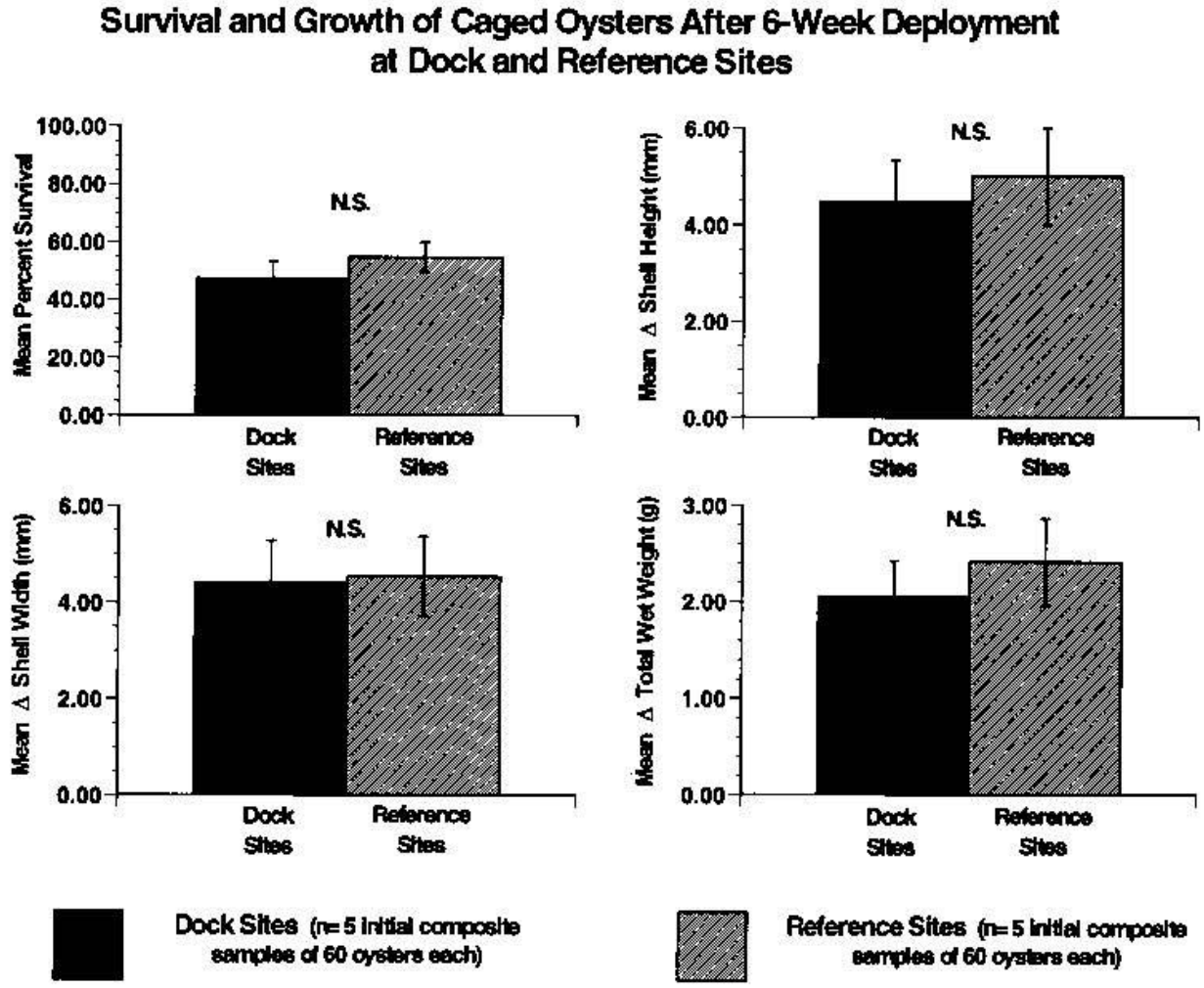

Figure 12. Mean (± 1 standard error) percent survival and growth of caged oysters after a 6-week deployment near newly constructed docks and reference sites.

The exceptionally low percent survival of *P. setiferus* at both dock and reference sites (< 50%) may indicate a high sensitivity to the stresses imposed by caging, or to the periodic hypoxia (<2mg l^{-1} D.O.) which occurred occasionally in both creeks, particularly during nighttime low tides (this study, unpublished data). Its high mortality at reference sites in both creeks suggests that results for this species may be equivocal.

Six-Week Oyster Growth and Bioaccumulation Studies: After six weeks of field exposure, the survival and growth of caged oysters (as measured by mean changes in shell height, shell width, and total wet weight) were slightly lower at dock sites than at reference sites (Fig. 12); however, none of these differences was statistically significant (ANOVA, $p>0.05$). Percent survival at all sites was low (ranging from 33-67%), suggesting that the oysters were succumbing to some stress (or stresses) other than that imposed by the presence of docks.

Among those oysters surviving at the end of six weeks, mean change in shell height was 4.5 mm at the five dock sites and 5.0 mm at the five reference sites. These values represent an average increase in size of 13.4% and 15.0% at dock and reference sites, respectively. Average shell width increased by 4.4 mm or 19.0% at the dock sites, and by 4.5 mm or 19.6% at the reference sites. Mean total wet weight (including the shell) increased by 2.0 g or 32.9% at the dock sites, and by 2.4 g or 38.6% at the reference sites. There were no significant differences between dock and reference sites in mean concentrations of copper or arsenic in composite oyster tissue samples (ANOVA, $p>0.05$), and chromium concentrations were below detectable levels in oysters deployed at all sites (Fig. 13).

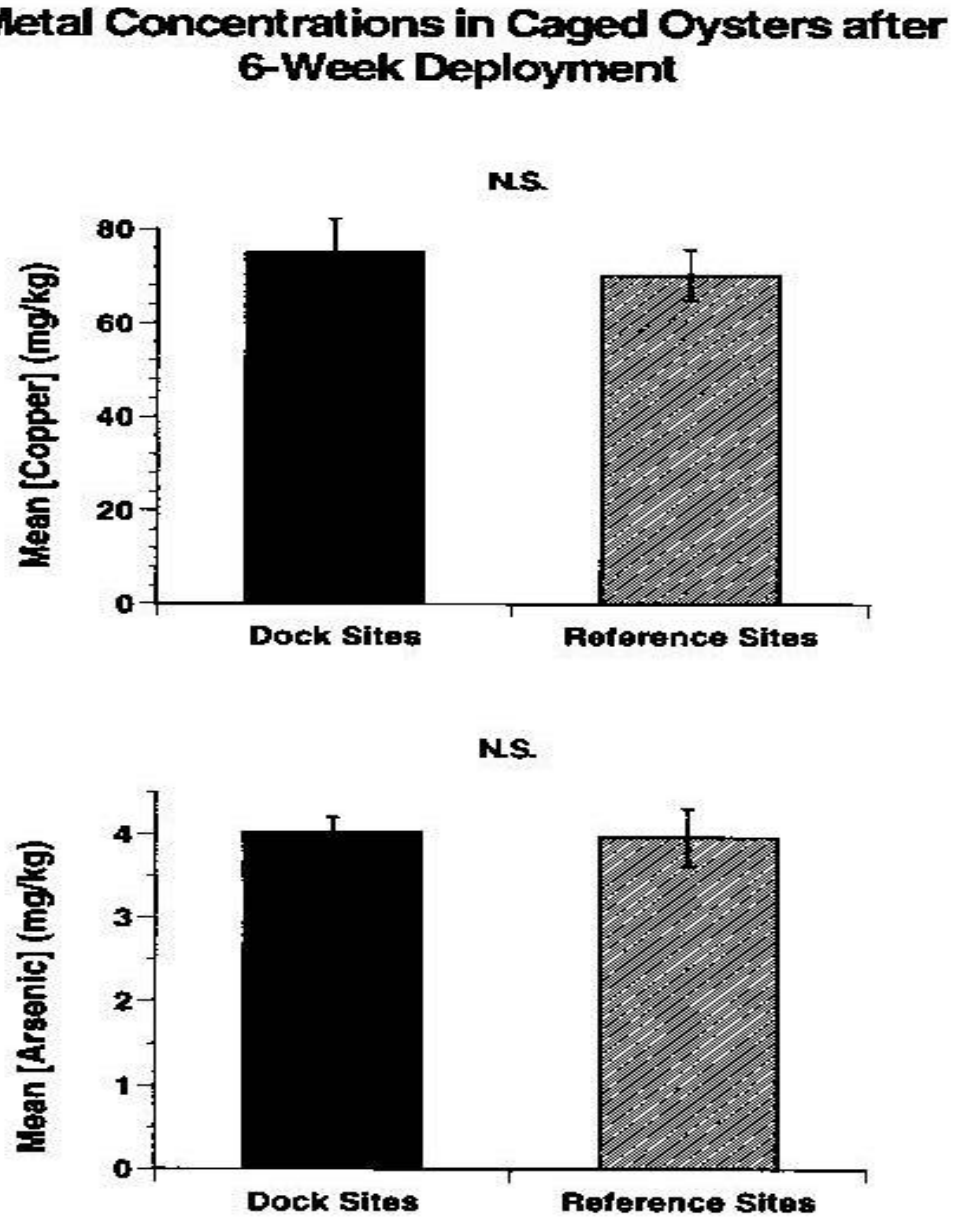

Figure 13. Copper and arsenic concentrations (ppm dry wt) in composite tissue samples of caged oysters after a 6-week deployment near newly constructed docks and reference sites; (chromium concentrations below detection limits at all sites).

DISCUSSION

In the first phase of our study, we examined our data for evidence of chronic exposure to wood preservative leachates in creeks with high densities of docks. Throughout our study area, concentrations of copper, chromium, and arsenic in tidal creek sediments were generally within the range of natural background levels, regardless of dock density or proximity. The most striking exception to this trend was the high copper concentration (>400 ppm dry wt) measured in one composite sediment sample from sites immediately adjacent to dock pilings in James Island Creek. Four additional sites in creeks with high dock densities had slightly elevated levels of arsenic. Weis et al. (1993c) found that sediments immediately adjacent to CCA-treated bulkheads had high concentrations of copper, chromium and arsenic associated with the fine-grained (i.e., silt/clay) fraction, but had very low percentages of these fine-grained sediments as a result of scouring. Conversely, silts and clays were more abundant further away from bulkheads, but had lower concentrations of these three metals. The authors attributed the gradient in metal concentrations to the accumulation of metal leachates from bulkheads in the fine-grained sediment fraction, and noted that the highest concentrations of metals were found in poorly flushed residential canals. In our study, percentages of silts and clays did not differ significantly between sites near (<1 m) and away (>10 m) from docks, suggesting that scouring around dock pilings was not sufficient to have altered the sediment regime there, and any site-related differences in metal concentrations were probably independent of sediment type.

Unlike Weis et al. (1993c), we did not specifically measure metal concentrations in the silt/clay fraction alone. Instead, we attempted to eliminate biases due to sediment type by normalizing total concentrations of copper, chromium and arsenic with respect to the reference element aluminum. Aluminum occurs in the fine-grained sediment fraction in the form of aluminosilicate clays, which are the dominant metal-bearing phases in coastal and estuarine sediments throughout the southeast (Schropp et al., 1990; Windom et al., 1989). Aluminum has historically been used as a normalizer of trace metal concentrations because it is naturally abundant and not typically associated with anthropogenic inputs (Windom et al., 1989). The absence of any significant difference in normalized metal concentrations between tidal creeks with and without high densities of docks, suggests that wood preservative leachates are not a significant source of metal contamination in sediments of well-flushed tidal creeks. This finding is consistent with Weis et al.'s

(1993c) observation of lower metal concentrations in open water environments compared to heavily bulkheaded canals. It should be noted, however, that likelihood of our detecting a significant difference in mean metal concentrations among sediments from different site groups was low, given the small magnitude of that difference for chromium and arsenic, and the large within-group variance-to-mean ratio observed for copper. This was reflected in the low power of all our statistical comparisons among site groups. Power values ranged from 0.12 to 0.30 (on a scale of 0 to 1) for the three analyses of covariance performed on copper, chromium and arsenic. These results indicate that there may have been site-related differences in metal concentrations that our sampling design was unable to detect. Nevertheless, the fact that all but one sediment sample had metal concentrations that were below levels known to cause biological effects suggests that any such statistical difference may not be ecologically significant. Furthermore, *a posteriori* analyses of sample size indicated that, depending on the metal, two to five times as many sediment samples would have had to be collected and analyzed (at substantially greater cost) in order to be 80% certain of detecting a significant difference in mean metal concentrations among site groups (at $p \leq 0.05$).

Metal and total PAH concentrations in whole sediments were generally below Long and Morgan's (1990) "ER-L" ("Effects Range - Low") levels, suggesting that concentrations of these substances at most sites were insufficient to cause toxic effects. This finding was substantiated by the results of our rotifer and Microtox bioassays, which showed no significant difference in the acute toxicity of bulk sediments or pore water between sites near to and distant from docks. Although the rotifer *Brachionus plicatilis* has been shown to be more sensitive to copper than it is to several other toxicants (Snell and Persoone, 1989), it experienced no substantial mortality when exposed to pore water from any of our sediment samples, even those with the highest dry weight copper concentrations. This suggests that any copper in the sediment pore water was either below levels which are acutely toxic to *B. plicatilis*, or the copper existed in a form that was unavailable for uptake. Sediment toxicity as measured by decreases in the bioluminescense of *Photobacterium phosphoreum* was positively associated with copper and arsenic concentrations, as well as dock density (no. docks/km shoreline). Dock density was not correlated with concentrations of either of these two metals, however. These findings suggest that, while high numbers of docks are not necessarily indicative of elevated metal concentrations in tidal creek sediments, they may be indicative of other anthropogenic stresses related to the density of adjacent residential development.

Unlike sediments, oysters collected from dock pilings contained significantly higher concentrations of copper than oysters collected from natural intertidal beds elsewhere in the same creeks or from reference creeks. There were no apparent trends in tissue concentrations of chromium or arsenic with respect to dock proximity, however. These findings are consistent with the higher leaching rate of copper from CCA-treated piles, and the apparent tendency for copper (unlike chromium or arsenic) to continually migrate from the inner section to outer surface of treated wood pilings, even after extended periods of exposure in the marine environment (Brooks, 1994). The ability of oysters to concentrate copper in their tissues to several orders of magnitude beyond ambient levels has been well documented (Cunningham, 1979; Greig and Wenzloff, 1978; Pringle et al., 1968). In our study, copper concentrations in oysters from dock pilings were generally comparable to those reported for oysters collected from CCA-treated dock pilings in an open water environment, but were lower than those reported for oysters from a heavily bulkheaded canal where histopathological abnormalities were found (Weis et al., 1993a). The highest copper concentrations recorded in our study were comparable to those in oysters from some of the more highly contaminated sites in the National Status and Trends Program (NOAA, 1987), and equalled or exceeded Pringle and Shuster's (1967) recommended guidelines for maximum copper concentrations in shellfish intended for human consumption (500 ppb dry tissue wt).

Copper is an essential micronutrient for most living organisms, but can have a variety of toxic effects when present at elevated levels (Cheng, 1989; Frazier, 1976; Phelps and Hetzel, 1987; Weis et al., 1993b). The accumulation and toxicity of copper are determined by numerous intrinsic and extrinsic factors (Cunningham, 1979). The former include age, size, weight, reproductive condition, and heavy metal body burden attained by the organism. The latter include factors such as temperature, salinity, pH, ambient copper concentration, duration of exposure, chemical speciation, and interactions with different metals. In our study, there was no evidence that the physiological condition of oysters growing on dock pilings was impaired, despite the elevated levels of copper in their tissues. Roosenberg (1969) found an inverse correlation between condition index and copper concentrations in oysters near the outfall of a steam electric generating station; however, copper levels in that study substantially exceeded the maximum values reported in this study.

We also found no evidence of significant shell thinning among oysters growing directly on dock pilings. Shell thinning can make oysters more susceptible to disease, as well as predation by

oyster drills, boring sponges and blue crabs (Frazier, 1976). Frazier (1976) found that, after a 1-year exposure period, hatchery-reared oysters placed in a metal-contaminated environment had significantly thinner shells and significantly higher copper concentrations than control oysters placed in a pristine environment. Condition index did not differ significantly between sites, however. The copper concentrations reported in that study (up to 450 ppm dry wt) were comparable to the highest values reported in our study; however, we found no correlation between copper concentrations in oysters and either condition index or shell thickness. We did find significant correlations between both of these biological parameters and arsenic levels; however, neither of these trends was related to the presence of docks.

While Phase I of our study focused on measuring the effects of chronic exposure to leachates from docks of undetermined age and uncertain treatment history, Phase II was specifically designed to assess the acute toxicity of CCA-leachates from recently constructed (4-12 mo-old) docks. In this latter portion of our study, we found no evidence of acute toxicity due to leachate exposure for any of the four species tested; although, the high mortality of juvenile white shrimp at both dock and reference sites suggests that results for this species may be equivocal. None of the other three species (red drum, mummichogs, and mud snails) suffered significantly greater mortality or accumulated significantly higher concentrations of metals at dock sites than at reference sites. These findings suggest that docks which are at least four months old pose no threat of acute toxicity to certain life stages of three representative estuarine species; however, our results do not preclude the possibility of acute toxicity occurring around more recently constructed (i.e., <4 mo old) docks. Evidence presented in an unpublished review article prepared on behalf of the Western Wood Preservers Institute (Brooks, 1994) indicates that leaching from CCA-treated wood is most rapid during the first five or six days after installation in aquatic environments, and that leaching rates are halved on each successive day after immersion in water. These findings suggest that any acute toxicity from CCA-treated piles in natural estuarine environments is likely to be very short-lived, if it occurs at all.

It should also be noted that our 4-day bioassays did not address the possibility of lethal or sublethal effects of CCA leachates on other estuarine species, or on earlier life stages of the four species tested. For instance, although we found no significant mortality for adult mummichogs (*Fundulus heteroclitus*) exposed to treated pilings in the field, Weis et al. (1991) observed significant mortality among embryos of the same species when exposed to large pieces of treated

wood in aquaria. This discrepancy could be due to the greater sensitivity of younger organisms to certain toxicants, or to the possibility of greater toxicant exposure in the static renewal tests. Critics of the Weis's research maintain that the leachate concentrations to which the embryos were exposed were unrealistically high compared to natural intertidal environments (Breteler, 1992). Differences in leachate concentration and mode of exposure might also explain the discrepancy between our findings and those of Weis et al.(1991) with respect to the mud snail *Ilyanassa obsoleta*. While we found no significant mortality for this species in the field, Weis et al. observed reduced activity and death of the same species within 72 hrs of exposure to CCA leachates in the laboratory. In our study, snails were permitted to graze freely on the ambient sediments adjacent to dock pilings, but (unlike Weis et al.'s study) they were not provided with algae which had been exposed to treated wood in aquaria for several days. Despite these discrepancies in methodology, our findings suggest that any exposure to CCA leachates is probably much lower in well-flushed estuaries than that experienced by test organisms in Weis et al.'s (1991) static renewal laboratory experiments.

Finally, our study of survival, growth, and bioaccumulation of metals in hatchery-reared oysters showed little evidence of adverse effects from CCA leachates. Some researchers have suggested that elevated copper concentrations might explain the stunted growth of oysters in certain subtidal habitats (Burrell et al., 1981; Phelps and Hetzel, 1987); however, Frasier (1976) found no reduction in growth of hatchery-reared oysters placed in a copper-contaminated environment, even after one year of exposure. In our study, there was no evidence of metal accumulation in caged oysters after six weeks of exposure to newly constructed docks, nor were there any significant difference in the mean percent survival or growth of these oysters compared to reference sites.

In summary, our findings suggest that, in natural estuarine environments subject to normal tidal exchange, wood preservative leachates from dock pilings have no acutely toxic effects on four common estuarine species, nor do they affect the survival or growth of oysters over a six-week period. In some cases, metal leachates may accumulate in sediments and oysters immediately adjacent to pilings, but do not appear to become concentrated in sediments or oysters elsewhere in the same creeks.

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